Remarks

The Examiner has objected to the specification "because the pages are not numbered". A substitute specification is enclosed herewith pursuant to 37 CFR 1.125 containing numbered pages. The description of the drawings and the priority information in the specification have also been corrected and updated as per the Examiner's request. No new matter has been added.

The Examiner has also requested a substitute sequence listing which is enclosed herewith to replace the sequence listing. Please replace the sequence listing appearing on original pages 47-48 of the application as filed with page 1 of the sequence listing filed herewith.

Claims 1 and 32-51 are currently pending. Claims 2-31 are canceled. Claims 1, 33-39, 41, 45, 46, and 49 have been amended to more distinctly describe the claimed subject matter. New claim 52 has been added. The spelling of "transgenic" in claim 38 has been corrected. The spelling of "blepharitis" in claim 35 has been corrected. No new matter has been added. Support for the amendments can be found throughout the specification and claims as originally filed. Specifically, support for the phrase "the phenotype of the mouse is characterized by increased Th2 cytokine production" can be found at least on page 9, lines 9-10. Support for the phrase "immune cell activation via a pathway that does not involve NFATp or NFAT4" can be found at least on page 10, lines 32-34. Support for the phrase "cells obtained from a mouse" can be found at least on page 11, line 7. Support for the phrase "exogenous DNA molecule" can be found at least on page 7, line 5. Support for the phrase "reduced FasL expression" can be found at least on page 39, lines 10-11. Support for the phrase "administering said test compound to a first transgenic mouse" can be found at least on page 10, lines 35-39. Support for the phrases "introducing an exogenous DNA molecule comprising at least a portion of NFATp gene into a mouse embryonic stem cell such that the wild-type NFATp gene of the embryonic stem cell is functionally disrupted" and "introducing said transgenic mouse embryonic stem cells into a pseudopregnant mouse such that said pseudopregnant mouse produces progeny comprising a functionally disrupted NFATp gene" can be found at least on page 9, lines 38-, page 10, lines 1-8. Support for the phrase "mating said

progeny with a functionally disrupted NFATp gene with said progeny with a functionally disrupted NFAT4 gene and identifying subsequent progeny with both a functionally disrupted NFATp gene and a functionally disrupted NFAT4 gene" can be found at least on page10, lines 20-22.

Claim Rejections Under 35 USC §101

Claims 1 and 32-51 have been rejected under 35 USC §101 because the Examiner asserts that the claimed invention is not supported by either a specific or substantial asserted utility or a well-established utility. More specifically, the Examiner states, "[t]he art since the time of filing does not teach the mice are a model for disease or teach how to use the mice to screen compounds", citing Oukka (*Immunity* 1998 9:295) and Hodge (*Immunity* 1996 4:397). Oukka teach the phenotypic characterization of mice with a NFAT4 disruption while Hodge teach the phenotypic characterization of mice with a NFATp disruption. As the Examiner states, since the time of filing of the instant invention, Rengarajan (*Immunity* 2000 12:293) and Ranger (*Immunity* 1998 9:627) teach mice with a disruption in both NFAT4 and NFATp but do not teach the mice as a model for disease.

The Examiner further states that "using a mouse already having increased Th2 activity to find compounds that increase Th2 activity is not specific to that mouse because wild-type mice can be used to find compounds that increase Th2 activity" and "using the mouse to identify compounds that regulate Th2 activity is not specific to that mouse, and the mouse claimed does not have a use that is specific to any disease". The Examiner continues, "[t]herefore compounds that modulate NFATp or NFAT4 cannot be found using the mice because NFATp and NFAT4 are not expressed in the mice".

Applicants respectfully traverse this rejection.

The pending claims are directed to methods of identifying compounds that modulate immune cell activation via a pathway that does not involve NFATp or NFAT4 utilizing ***cells obtained from a mouse deficient in NFATp and NFAT4 or mice comprising a genome deficient in NFATp and NFAT4*** and to methods of producing ***transgenic mice lacking NFATp and NFAT4.*** As the claims all require the use of cells or animals lacking NFATp and NFAT4, the use of wild-type animals that normally do not have increased Th2 activity is not embraced by the claims. Furthermore, wild-type

animals that possess an intact system to repress Th2 activity, *e.g.*, intact NFATp and NFAT4 genes, would not be useful to identify compounds that modulate immune cell activation via a pathway that does not utilize NFATp and/or NFAT4.

The present invention is based on the surprising discovery that mice deficient in both NFATp and NFAT4 exhibit features characteristic of profound increases in Th2 cell activity, thus demonstrating that NFATp and NFAT4 are required for the control of lymphocyte homeostasis and act as selective repressors of Th2 cells. Based on these discoveries, the present invention provides methods to identify agents that modulate immune cell activation via a pathway that does not involve NFATp or NFAT4, utilizing cells and animals deficient in NFATp and NFAT4.

In contrast to the Examiner's assertion that the present invention is directed towards methods to identify compounds that increase Th2 activity in mice that already have increased Th2 activity (whether or not NFATp and NFAT4 have been disrupted, *e.g.*, wild-type mice), Applicants direct the Examiner to page 10, lines 28-34 which particularly points out that the methods of the present invention are for ***identifying agents that modulate Th2 cell activity by means other than modulating NFATp or NFAT4 themselves***:

> In one embodiment, the invention provides methods for identifying compounds that modulate Th2 cell activity using cells deficient in NFATp and NFAT4. As described in the Examples, inhibition of NFATp and NFAT4 activity (*e.g.*, by disruption of both the NFATp and NFAT4 genes) leads to greatly increased Th2 cell activity. Accordingly, lymphoid cells from NFATp/NFAT4 doubly deficient mice, having enhanced Th2 cell activity, can be used to identify agents that modulate Th2 cell activity by means other than modulating NFATp or NFAT4 themselves.

To further clarify this point for the Examiner, Applicants have amended claims 1 and 45 to recite that the methods are directed to the identification of compounds that modulate immune cell activation via a pathway that does not involve NFATp and NFAT4.

Accordingly, the doubly transgenic animals of the invention have a specific utility, *e.g.*, the identification of a test compound that modulates immune cell action via a pathway that does not involve NFATp and NFAT4, utilizing mice or cells obtained from a mouse deficient in NFATp and NFAT4. Accordingly, Applicants request

reconsideration and withdrawal of the rejection of claims 1 and 32-51 under 35 USC §101.

Claim Rejections Under 35 USC §112, First Paragraph

Claims 1, 32-51 have been rejected under 35 USC §112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner states that the claimed invention has no specific utility and therefore one skilled in the art would not know how to use the claimed invention.

The Examiner states that claim 1 encompasses wild-type phenotype. Applicants respectfully traverse this rejection. As set forth above, the pending claims do not embrace the use of wild-type cells or animals. By definition, any animal deficient in any two genes, *e.g.*, NFATp and NFAT4, is not wild-type. "Wild-type" refers to the genotype or phenotype that is found in nature or in the standard laboratory stock for a given organism. The claims also expressly indicate that the transgenic animals or cells derived therefrom have a non-wild-type phenotype, *e.g.*, one characterized by increased Th2 cytokine production.

The Examiner has rejected claims 50 and 51 because they encompass mice and rats. Claims 50 and 51 have been amended to recite "transgenic mouse cell", thereby rendering the objection moot.

The Examiner has rejected claim 49, stating "[t]he claim does not require using mouse ES cells. Claim 49 has been amended to recite "mouse embryonic stem cells" rendering the rejection moot.

The Examiner has also rejected claim 49 because "the specification does not teach introducing both constructs into the ES cell at the same time". Claim 49 has been amended to specify that two separate transgenic mice are produced and subsequently mated to produce the double transgenic mouse of the invention. In addition, new claim 52 has been added and is directed to an embodiment of the invention where two single knockout mice are mated to produce a double knockout mouse.

The Examiner has also rejected claim 49 because the "claim does not require the mouse have a phenotype that differs from wild-type". As stated above, the claims have been amended to recite the phenotype of the mice.

The Examiner has rejected claims 45-48 because specific guidance is not provided to determine compounds that modulate NFATp and NFAT4. Applicants respectfully traverse and reiterate that the pending claims are not directed toward the identification of modulators of NFATp and NFAT4, but rather ***modulators of immune cell activation via a pathway that does not involve NFATp and NFAT4***. In addition as discussed below, support for claim 45 can be found at least on page 10, lines 37-, page 11, lines 1-2 which teaches that "[m]odulation of Th2 cell activity of the NFATp/NFAT4 deficient lymphoid cells (as compared to an appropriate control such as, for example, untreated cells or cells treated with a control agent) identifies a test compound as a modulator of Th2 cell activity.

Claims 32-51 are rejected because "[t] specification as originally filed did not support the breadth of any 'nucleic acid molecule' that functionally disrupts an NFATp or NFAT4 gene". Solely in the interest of expediting prosecution, the claims have now been amended to provide that a "DNA molecule" disrupts the genes in claim 32 and 49, and dependent claims 33-44 and 50-51, rendering the rejection moot.

The Examiner states that the phenotypes in new claims 33-44 cannot be found in Examples 1-3 or on page 36-41 as stated in the preliminary amendment.

Claim 33 is directed toward a double transgenic mouse with a phenotype characterized by lymphadenopathy, or swelling of the lymph nodes. This characteristic of the double transgenic mouse of the present invention is described in Example 1, on page 37, lines 36-, page 38, lines 1-9:

> DKO mice exhibited massive ***splenomegaly*** and ***lymphadenopathy*** by 7 weeks of age. Histological analysis of the spleen and lymph node revealed disruption of the normal architecture by numerous granulomas. The architecture of the lymph node and spleen of the DKO is disrupted by granulomatous lesions containing multinucleated giant cells. There was also a marked increase in mast cells in DKO spleen. Toluidine-blue stained spleen sections from wild-type and DKO mice showed numerous mast cells identified by intense staining of intracellular granules. The absence of multiorgan lymphoid infiltration and immune complex-mediated pathology distinguishes the NFAT DKO from other mouse

> strains that display massive lymphadenopathy such as CTLA-4 and IL-2 receptor alpha deficient and TRAF2 dominant negative mutant mice [Tivol, E.A. et al., *Immunity* 3:541 (1995); Waterhosue, P. et al., *Science* 270:985 (1995); Sadlack, B. et al., *Eur. J. Immunol.* 25:3053 (1995); Willerford, D.M. et al., *Immunity* 3:521 (1995)]. This is consistent with the normal protein or RNA levels of these genes in NFAT DKO lymphocytes. (emphasis added)

Claim 34 is directed toward a double transgenic mouse with a phenotype characterized by splenomegaly, or an enlargement of the spleen. This characteristic of the double transgenic mouse of the present invention is described in Example 1, on page 37, lines 36-, page 38, lines 1-9, as above.

Claim 35 is directed toward a double transgenic mouse with a phenotype characterized by blepharitis, or inflammation of the eyelids. This characteristic of the double transgenic mouse of the present invention is described in Example 1, on page 37, lines 25-30:

> DKO mice demonstrated modest growth retardation and developed severe bilateral ***blepharitis*** by approximately 4 weeks after birth. Histological evaluation of the eye and the surrounding tissues revealed a complex cellular infiltrate composed of lymphocytes, macrophages, mast cells and plasma cells. In all DKO animals examined (n=5), the eyelids displayed edema and ulceration with underlying granulation tissue and a marked inflammatory infiltrate. (emphasis added)

Claim 36 is directed toward a double transgenic mouse with a phenotype characterized by interstitial pneumonitis which is marked by diffuse peribronchial and interstitial infiltration of the lungs by lymphocytes, plasma cells, and lymphoblasts. This characteristic of the double transgenic mouse of the present invention is described in Example 1, on page 37, lines 30-33:

> Examination of the lungs revealed an acute and chronic ***interstitial pneumonitis*** characterized by an intense inflammatory infiltrate consisting of lymphocytes, plasma cells, neutrophils and mast cells or basophils. The inflammatory infiltrate surprisingly did not include eosinophils. (emphasis added)

Claim 37 and dependent claim 38 are directed toward a double transgenic mouse with a phenotype characterized by an increase in peripheral T cells with a

memory/activated phenotype. This characteristic of the double transgenic mouse of the present invention is described in Example 1, on page 38, lines 24-28:

> In the absence of NFATp and NFAT4 there was a dramatic increase in the percentage of peripheral T cells with a ***memory/activated phenotype*** as indicated by low levels of Mel-14 and CD45RB and elevated levels of CD44 and CD69 on spleen cells and LN. The activated/memory cells did not represent a clonal expansion of T cells as evaluated by their Vβ and Vα usage. (emphasis added)

Claim 39 and dependent claim 40 are directed toward a double transgenic mouse with a phenotype characterized by compromised FasL expression leading to defective apoptosis. This characteristic of the double transgenic mouse of the present invention is described in Example 2, beginning on page 38, lines 30-, page 39, lines 1-28.

Claim 41 and dependent claims 42- 43 are directed toward a double transgenic mouse with a phenotype characterized by increased Th2 cytokine production. This characteristic of the double transgenic mouse of the present invention is described in Example 3, page 39, lines 31- page 41, lines 1-5.

The Examiner states that the steps of claim 45 (and dependent claims 46-48) are not disclosed in Examples 1-3 and the "specification does not teach administering a compound to one mouse but not the other or comparing the 'Th2 cell activity' of each mouse".

Applicants respectfully traverse this rejection and point the Examiner to page 10, lines 37-, page 11, lines 1-5, which describes the method of claim 45 and states:

> In the screening method, lymphoid cells deficient in NFATp and NFAT4 are contacted with a test compound and ***Th2 activity of the lymphoid cells is monitored. Modulation of Th2 cell activity of the NFATp/NFAT4 deficient lymphoid cells (as compared to an appropriate control such as, for example, untreated cells or cells treated with a control agent) identifies a test compound as a modulator Th2 cell activity***. In one embodiment, the test compound is administered directly to an NFATp/NFAT4 deficient mouse to identify a test compound that modulates *in vivo* Th2 cell activity. In another embodiment, lymphoid cells deficient in NFATp and NFAT4 are isolated from the

> NFATp/NFAT4 deficient mouse, and are contacted with the test compound *ex vivo* to identify a test compound that modulates Th2 cell activity. (emphasis added)

And page 13, lines 17-24:

> Following contact of the NFATp/NFAT4 deficient lymphoid cells with a test compound (either *ex vivo* or *in vivo*), the effect of the test compound on Th2 cell activity can be determined by any one of a variety of suitable methods, including monitoring of Th2-associated cytokine production or IgG1 and/or IgE production. Examples of such methods are described in detail in the Examples. A test compound is identified as a modulator of Th2 cell activity based on its ability to modulate Th2 cell activity of NFATp/NFAT4 deficient lymphoid cells, as compared to an appropriate control (such as untreated cells or cells treated with a control compound, or carrier, that does not modulate Th2 cell activity).

In addition, claim 45 has been amended to clarify that the second mouse receives an appropriate control.

The Examiner states that the amino acid sequences of SEQ ID NOs.: 1-3 are not found, and that the administration of "the amino acids to mice as claimed was not contemplated in the specification".

Applicants point the Examiner to, at least, page 29, lines 15-38:

> ***In another embodiment, an inhibitory compound of the invention is a peptidic compound derived from the NFATp and/or NFAT4 amino acid sequence.*** In particular, the inhibitory compound(s) comprises a portion of NFATp and/or NFAT4 (or a mimetic thereof) that mediates interaction of NFATp/NFAT4 with a target molecule such that contact of NFATp/NFAT4 with this peptidic compound competitively inhibits the interaction of NFATp with the target molecule. ***In a preferred embodiment, the peptide compound is designed based on the region of NFATp/NFAT4 that mediates interaction of NFATp/NFAT4 with calcineurin.*** As described in Avramburu *et al.*, (1998) *Mol. Cell.* 1:627-637 (expressly incorporated herein by reference), a conserved region in the amino terminus of NFAT proteins mediates interaction of the NFAT proteins with calcineurin and peptides spanning the region inhibit the ability of calcineurin to bind to and phosphorylate NFAT proteins, without affecting the phosphatase activity of calcineurin against other substrates. Moreover, when expressed intracellularly, peptide spanning this region inhibits NFAT dephosphorylation, nuclear translocation and NFAT-mediated gene expression in response to stimulation, thereby inhibiting

> NFAT-dependent functions. The region of NFATp mediating interaction with calcineurin contains the conserved amino acid motif: ***Ser-Pro-Arg-Ile-Glu-Ile-Thr (SEQ ID NO:1).***
>
> In a preferred embodiment, a NFAT inhibitory compound is a peptidic compound, which is prepared based on a calcineurin-interacting region of NFATp. A peptide can be derived from the calcineurin-interacting region of NFATp having an amino acid sequence that comprises the 9 amino acid motif of SEQ ID NO: 1. Alternatively, longer regions of human NFATp can be used such as a peptide that comprises the 25 amino acids of ***SEQ ID NO: 2*** (which spans the motif of SEQ ID NO: 1) or the 13 amino acids of ***SEQ ID NO: 3*** (which also spans the motif of SEQ ID NO: 1). (emphasis added)

In addition, Applicants point the Examiner to page 21, lines 12-14:

> Exemplary compounds which can be screened for activity include, but are not limited to, ***peptides,*** nucleic acids, carbohydrates, small organic molecules, and natural product extract libraries.

The nucleotide sequences that correspond to SEQ ID Nos.:1-3 can be found in the specification as originally filed on pages 47-48 following the abstract. The nucleotide sequences that correspond to SEQ ID Nos.:1-3 can also be found in the new sequence listing that is submitted herewith.

Claim 49 is rejected because the "steps cannot be found". Applicants respectfully traverse this rejection. Claim 49 is directed to methods of producing a double transgenic animal, a technique known in the art. This technique includes the steps of creating a transgenic embryonic mouse stem cell by introducing exogenous DNA into the mouse ES cell and functionally disrupting the endogenous gene of interest. The transgenic ES cells are introduced into pseudopregnant females to produce progeny carrying a disrupted gene and then are mated to produce a transgenic mouse line. These methods are generally described in, Section I. NFATp/NFAT4 Deficient Mice, beginning on page 9 of the present specification.

Claim 50 is rejected because the "breadth of 'murine' was not contemplated". Claim 50 has been amended to recite "a transgenic mouse cell", thereby rendering the Examiner's rejection moot.

Claim 51 is rejected because the types of cells were not contemplated. Applicants point the Examiner to the following pages for support for the cells in claim 51:

Page 9, lines 38-, page 10, line 1:

> The vector is introduced into an ***embryonic stem cell line (e.g., by electroporation) and cells in which the introduced NFATp or NFAT4 gene has homologously recombined with the endogenous NFATp or NFAT4*** gene are selected.

Page 10, lines31-34:

> Accordingly, ***lymphoid cells from NFATp/NFAT4 doubly deficient mice,*** having enhanced Th2 cell activity, can be used to identify agents that modulate Th2 cell activity by means other than modulating NFATp or NFAT4 themselves.

And page 10, lines 6-8:

> Progeny harboring the homologously recombined DNA in their ***germ cells can be used to breed animals*** in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene.

In light of the above stated arguments and amendments, Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 1 and 32-51 under 35 USC §112, first paragraph.

Claim Rejections Under 35 USC §112, Second Paragraph

Claims 1 and 32-51 have been rejected under 35 USC §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Examiner states that the term "'exogenous' in claims 1, 32 and 49 is indefinite because the "term is relative". Applicants respectfully traverse this rejection and point to the definition of "exogenous DNA" on page 9, lines 7-10 which states, "[t]he term 'exogenous DNA' refers to a DNA molecule that does not naturally occur in that location of the genome of the mouse and that serves to disrupt the natural endogenous gene". Applicants argue that this definition is a term used in the art, and

although broad, is not indefinite and covers DNA, *e.g.*, NFATp and NFAT4 transgenes, which are exogenous to mice as a species and the mouse as an individual.

The Examiner also rejects claims 33-36 because it is unclear if the term "characterized" means the mouse has the trait or has the qualities of the trait. Applicants respectfully traverse and argue that the use of the word "characterized" is definite based on the common definition: to make distinct and recognizable by peculiar marks or traits; to make with distinctive features (from www.Dictionary.com). Claims 33-36 recite that the transgenic mice of the invention have a phenotype "characterized by", *e.g.*, lymphadenopathy, splenomegaly, blepharitis, interstitial pneumonitis, relative to wild-type mice which makes the transgenic mice of the invention distinct and recognizable relative to a normal, control, *e.g.*, wild-type, mouse.

The Examiner also rejects claim 38 because "the metes and bounds of what applicants consider 'memory/activated phenotype' cannot be determined." Applicants respectfully traverse and point the Examiner to page 38, lines 23-28 which specifically teaches that memory/activated T cells are identified based on low levels of Mel-14 and CD45RB and elevated levels of CD44 and CD69:

> In the absence of NFATp and NFAT4 there was a dramatic increase in the percentage of peripheral T cells with a memory/activated phenotype as indicated by low levels of Mel-14 and CD45RB and elevated levels of CD44 and CD69 on spleen cells and LN. The activated/memory cells did not represent a clonal expansion of T cells as evaluated by their Vβ and Vα usage.

The Examiner also rejects claim 39 because it is unclear if the claim is "limited to decreased FasL expression or whether the claim encompasses normal FasL expression level of a compromised FasL protein". Applicants have amended claim 39 to recite that FasL expression is reduced relative to a wild-type mouse, thereby rendering the Examiner's rejection moot.

The Examiner also rejects claim 43 because "the metes and bounds of what applicant's consider 'IL-4 dependent immunoglobulin isotypes' cannot be determined". Applicants respectfully traverse this rejection and point the Examiner to page 6, lines 10-

13 which teaches that IL-4 dependent immunoglobulin isotypes are those that are associated with efficient B cell help such as IgG1 and IgE, two isotypes known in the art to provide that help to B cells:

> As used herein, the term "Th2 cell activity" refers to activity of a subpopulation of $CD4^+$ T cells that is characterized by the production of one or more cytokines selected from IL-4, IL-5, IL-6, IL-10 and IL-13, and that is associated with efficient B cell "help" provided by the Th2 cells (*e.g.*, enhanced IgG1 and/or IgE production).

The Examiner also rejects claim 45 because "the metes and bounds of what applicants consider 'calcineurin-interacting region of NFATp and NFAT4' cannot be determined". Applicants believe the Examiner is referring to claim 46 and not claim 45, therefore claim 46 is addressed below.

Applicants respectfully traverse this rejection and point the Examiner to page 29, lines 22-31 which specifically teaches that the region of NFATp and NFAT4 that interacts with calcineurin is known in the art and contains the conserved amino acid motif: Ser-Pro-Arg-Ile-Glu-Ile-Thr:

> As described in Avramburu *et al.*, (1998) *Mol. Cell.* 1:627-637 (expressly incorporated herein by reference), a conserved region in the amino terminus of NFAT proteins mediates interaction of the NFAT proteins with calcineurin and peptides spanning the region inhibit the ability of calcineurin to bind to and phosphorylate NFAT proteins, without affecting the phosphatase activity of calcineurin against other substrates. Moreover, when expressed intracellularly, peptide spanning this region inhibits NFAT dephosphorylation, nuclear translocation and NFAT-mediated gene expression in response to stimulation, thereby inhibiting NFAT-dependent functions. The region of NFATp mediating interaction with calcineurin contains the conserved amino acid motif: Ser-Pro-Arg-Ile-Glu-Ile-Thr.

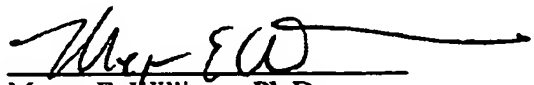
The Examiner also rejects claim 48 because "said peptidic compound" lacks antecedent basis. Applicants have amended claim 48 to recite "test compound" thereby rendering the Examiner's rejection moot.

In light of the above stated arguments and amendments, Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 1 and 32-51 under 35 USC §112, second paragraph as being indefinite.

CONCLUSION

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,



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